BULLETIN No. 19. V. P. P.—71.

U. S. DEPARTMENT OF AGRICULTURE.
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.
B. T. GALLOWAY, Chief.

STIGMONOSE:

A DISEASE OF CARNATIONS AND OTHER PINKS.

BY

ALBERT F. WOODS,
ASSISTANT CHIEF DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Division of Vegetable Physiology and Pathology,
Washington, D. C., February 15, 1900.

Sir: I respectfully transmit herewith a report by Mr. Albert F. Woods, of this Division, embodying the results of an investigation of a disease of the carnation, to which he has applied the name "stigmonose." This disease, which is a serious drawback to the successful growing of the carnation, has until recently been attributed to bacteria and given the name "bacteriosis." Mr. Woods, however, has shown that it is due to the punctures of insects, principally aphides and thrips, and of mites. Although these are the inciting cause of the trouble, the pathological and physiological changes involved are dependent to a large degree on the condition of the plant and the time the punctures are made. The work deals with the subject largely from the standpoint of pathology and physiology, pointing out the conditions which influence the disease, and suggesting lines of treatment based mainly on a knowledge of the proper handling of the plants. We are indebted to Dr. L. O. Howard, Entomologist of the Department, for assistance in a number of matters connected with the work.

The carnation crop in this country represents an annual value of over $4,000,000 and is constantly increasing. In view of this fact and the many interesting and suggestive points brought out in the work, I respectfully recommend that the report be published as Bulletin No. 19 of this Division.

Respectfully,

B. T. Galloway,
Chief of Division.

Hon. James Wilson,
Secretary of Agriculture.

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STIGMONOSE: A DISEASE OF CARNATIONS AND OTHER PINKS.

INTRODUCTION.

In the course of some investigations which the writer conducted several years ago on a disease of the Bermuda lily, his attention was attracted by the similarity between this disease and the one affecting carnations, and described by Arthur and Bolley[1] under the name "bacteriosis." As announced in a preliminary paper,[2] the writer carried on extended studies of the carnation disease, but was unable to obtain results similar to those described by Arthur and Bolley. Since publishing the paper referred to he has repeated and extended the work which it describes and has fully substantiated his former conclusions. In view of the fact that the disease is not due to bacteria, but is caused by the punctures of aphides, thrips, and occasionally of red spiders, the name stigmonose,[3] or puncture disease, is here suggested as an appropriate one for the malady.

Probably the first account of the disease was that given in Dr. Arthur's paper[4] read before the American Association for the Advancement of Science, at its meeting at Toronto, in 1889. In this paper Dr. Arthur proposed the name "bacteriosis," believing the disease to be due to bacteria.

Inasmuch as many pathologists and carnation growers remain in doubt regarding the true nature of the trouble, it is thought desirable at this time to give a full account of all the work that has been done on it, together with a detailed discussion of the conclusions reached by Arthur and Bolley.

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[1] Arthur, J. C., and Bolley, H. L., Purdue Univ. Exp. Sta. Bull. No. 59, 1896, Vol. VII, pp. 29, pls. 8.

[2] Bot. Gaz., September, 1897, and Centralbl. f. Bakt., Par., u. Infekt., 1897, 2 Abt., Bd. 3.

[3] Stigmonose is a combination of the two Greek words στιγμή, a puncture, and νόσος, a disease, the puncture, rather than the organism producing it, being made the basis of the name.

[4] Arthur, J. C., Proc. Am. Assn. Adv. Sci., 1889, Vol. XXXVIII, p. 280.

INVESTIGATIONS OF THE CARNATION DISEASE BY ARTHUR AND BOLLEY.

DESCRIPTION OF THE DISEASE.

The trouble was regarded by Arthur and Bolley[1] as one affecting the carnation leaf mainly. They describe it as follows:

It generally starts in the leaf when immature, and is best diagnosed in the younger but full-sized leaves nearest the upper end of the stem. Taking such a leaf, which on its surface presents no unusual appearance to the eye, and holding it toward a strong light, small, pellucid dots may be detected scattered irregularly through the leaf, sometimes having a faint yellowish color, which are the centers of infection. The appearance of the dots has a close resemblance to those of the oil glands in the leaves of the common St. John's wort (*Hypericum perforatum*), a rather abundant weed, or in the leaves of the false indigo (*Amorpha fruticosa*), a native shrub, except that they have no regular disposition. Sometimes the surface of the leaf is slightly raised over the dots, making watery pimples.

After a time the surface of the leaf above the dots changes enough to indicate their presence and finally shows a distinct spot. As the disease extends inside the leaf the surface tissues dry, the internal tissues collapse, and whitish, sunken spots appear. In some colored varieties of carnation the spots vary somewhat by being more or less reddish or purplish. As the spots increase in size the leaves wither, still clinging to the stem. Such spots never show distinct central darker-colored specks and rarely any concentric circles, as do the spots made by parasitic fungi, such as Septoria (spot disease) and Heterosporium (fairy ring).

Very badly diseased plants, especially when much crowded and growing in damp atmosphere, have more yellowish green leaves than normal, of a more transparent appearance, and usually smaller. The lower leaves of diseased plants in any atmosphere or soil die prematurely and the vitality of the plant is so lowered as to check the growth and decrease the size and number of the flowers.

In addition to the above description, these translucent dots may be divided into three classes, representing three forms of the disease, which differ slightly from each other in general appearance and behavior. The first and most common form corresponds with the above description. The translucent dots increase more or less rapidly, according to the age of the leaf, until finally they reach a diameter of one to several millimeters, and often coalesce. The second form is characterized by the appearance of very small spots, scarcely visible to the naked eye, but easily detected when the leaf is held up to the light and examined with an ordinary hand lens. These small spots are exceedingly numerous, but in their further development, even on young leaves, scarcely ever reach a diameter of more than half a millimeter. The third form resembles the first in extent of development, but instead of being round, the spots are elongated and irregular, and the affected plants are usually more or less distorted.

Dr. Arthur said that in his earlier study of the disease "no cause, either parasitic or nonparasitic, could be found to account for the difficulty. The eye, either unaided or by the assistance of the microscope,

[1] Arthur, J. C., and Bolley, H. L., Purdue Univ. Exp. Sta. Bull. No. 59, 1896, p. 18. All succeeding quotations from these authors are from this bulletin.

detected only a gradual shriveling of the tissues of the leaves. At first light-colored blotches made their appearance, which gradually extended, coalesced, and finally the whole leaf became dry and lifeless." A further examination made by Dr. Arthur in 1889 revealed the presence of the semitransparent dots described. He says:

Repeated microscopic examinations of the sections through these spots convinced us that the cells of the region were always infested with bacteria—often in considerable numbers. It was not difficult to see that the conspicuous dry blotches might be derived from the inconspicuous dots and that the latter possibly represented the beginnings of a genuine disease. There were now a number of difficult questions to answer, foremost of which was to show the causal or accidental relation of the bacteria seen in the cells to the degeneration of the tissues. The larger part of the work of the investigation from this time on until the main facts were established—a period of about eighteen months—fell to the lot of Mr. Bolley.

ISOLATION OF THE GERM.

Following out the suggestions resulting from their microscopical examinations, Arthur and Bolley made an attempt to isolate the germ supposed to be the cause of the trouble. It should be noted that their "repeated trials for a number of months gave such varying results that nothing of a substantial nature was learned. Saprophytic forms from the air and from the leaves of the plant took possession of the cultures, obscuring or destroying the slower-growing parasitic form." However, the addition of malic acid to the culture media retarded the growth of saprophytic bacteria and gave the slower-growing organisms a chance to develop. One of the most constant sorts obtained by Arthur and Bolley[1] in this way was a "coccus-like form of a yellowish color that grew slowly, but developed well in acid cultures," and this they believed, as the result of infection experiments, to be the cause of the disease in question.

No spores were found in any stage of growth. The bodies discovered in the cells of the host and believed to be bacteria agreed in size and general appearance with *Bacterium dianthi*. The cells were not united into filaments, but were almost or quite separate from one another, exhibiting no independent movement and undergoing no marked variation in form during growth.

It is not necessary in this paper to enter into a full discussion of the biology of this germ, but a few of the most important points may be mentioned. In plate cultures of 10 per cent acid nutrient gelatin the body of the colony is made up largely of zooglœa, giving an "irregular

[1] They describe the species as "*Bacterium dianthi* Arthur and Bolley n. sp. Cells oval to elliptical, single or rarely united, 0.9–1.25 by 1–2 μ; in rich fluid media more united, in part forming short filaments, at first motile, afterwards forming distinct, elongated, somewhat convoluted zooglœa; on solid media becoming yellow in mass. * * * The color accumulates slowly as the bacteria grow and is apparently not deposited within the cell, but is an excretion from it."

outline and a lobed and wrinkled appearance to the surface, the color being a light, clear orange. When growth is strong the colonies pile up in pronounced yellow, viscid drops." In plate cultures of neutral gelatin the colonies consist after a time of a few light-colored zoogloœa, "with a surrounding irregular area of actively growing bacteria, the whole having a light cream color." The germ liquefies gelatin slowly. The formation of zoogloœa is also particularly marked in fluid culture media, such as a broth made of corn seedlings or of potato tubers. The germ stains readily in all stages of development with most of the dyes in general use, especially aqueous solution of fuchsin and of gentian violet.

DIFFICULTIES MET WITH IN ISOLATING THE ORGANISM.

As before stated, Arthur and Bolley found more or less difficulty in isolating the germ from the diseased tissues, even the acidifying of the gelatin only partially preventing rapidly growing organisms from overrunning the cultures. Attempts to free the surface of the leaves from other germs by washing with corrosive sublimate (1:1,000 solution) always gave negative results. They believed that "some of the poison passed over into the culture and prevented growth, even when the leaf was well washed with distilled water after its treatment with corrosive sublimate. Most of the work was done with cultures obtained by passing the leaf quickly through a Bunsen gas flame two or three times, thus destroying all surface molds and bacteria in both spore and vegetative condition, and then cutting the leaf into thin sections with flamed scissors, allowing the sections to drop into the nutrient medium."

The first point to be investigated was the adequacy of this method of surface sterilization. Numerous experiments by the writer have shown conclusively that unless the epidermal cells of the carnation are killed by the heat the organisms lodged in crevices of the cuticle are not always killed, but often grow readily when a portion of the flamed though still living epidermis is put into nutrient media. Flaming the leaf, therefore, in the manner described is sufficient to remove from it only the most exposed and least resistant organisms.

It is to be regretted that Arthur and Bolley did not describe the poured plate cultures made direct from the diseased tissues, as this is a much more accurate method of research than simply dropping fragments of tissue into tubes of culture media and then making poured plates from the growth thus obtained. If the diseased mesophyll cells contain even a few germs, hundreds of colonies ought to be obtained by breaking up these cells and making poured plates from them direct. If only a few colonies develop after the cells are broken up in quantity and such plates made, the direct evidence that the germ had anything to do with causing the disease would be very inconclusive, especially if the

colonies are of different varieties. However, if such plates should uniformly yield numerous colonies mostly of one sort, the evidence would be favorable, but still not conclusive.

INFECTION EXPERIMENTS.

Arthur and Bolley's first infection experiments were made with liquid cultures developed from cutting up diseased leaves into nutrient fluid. When the fluid became cloudy "application of the liquid was made with a camel's hair brush to the surface of four young leaves of a a small, healthy carnation plant growing in a greenhouse." After fifteen days indications of change were detected in the infected region, and in nineteen days the characteristic dots were seen where the infusion was applied, "the remainder of the plant remaining quite free from any such appearance." Another plant, covered by a bell jar, was similarly treated a few days later, and in seventeen days showed distinct evidences of the disease. Nothing is known with regard to the organisms which this culture may have contained.

After the isolation of the yellow germ described, many surface infections were tried, but, according to the authors, "the purity of the cultures could not always be guaranteed, or else the trial plants developed diseased spots outside the inoculated area and thus absolute certainty could not be obtained." The investigators named make the following statements in regard to their infection experiments:

January 15, 1890, an infection of three seedling carnations in the greenhouse, two protected with bell jars and one uncovered, was made as previously described, using a potato infusion. This infusion had been infected the day before with the yellow coccus-like germs from a pure culture on solid media, which had originally been obtained from diseased carnation leaves on November 18, 1889. The affected areas began to show disease in six days, and in eleven days more all the leaves became "infected at the points of application, and at these points only," as stated in the record book, and remained so for a month and a half or more. It was now believed that the germ causing the bacteriosis was found, and although many subsequent infection experiments were carried out with varying success, the remainder of the winter was chiefly given up to the biological study of the specific germ.

The best method for applying the contagium was for a time uncertain. The method used in studying pear blight, the well-investigated bacterial disease of pomaceous trees, which consists in abrading the surface so that the germs may at once come in contact with the internal juices and tender tissues of the plant, proved inapplicable to the carnation disease. No clearly marked cases of the disease were obtained in this way. The wounds showed in some cases a slightly yellowish margin, but otherwise gave few indications of results differing from those which might arise from accidental abrasions.

Surface application (wetting the uninjured surfaces of the young leaves with the germ-laden fluid) was finally adopted as the proper method of infection. Experience showed that success could only rarely be attained when the infectious fluid was applied to mature leaves. The best results were always secured when the application was made to the small appressed leaves at the end of the stem. They were drawn back and well wet with the fluid, and if growing vigorously usually showed the characteristic pellucid dots by the time the leaves were full size. A difficulty was

experienced in securing perfectly healthy plants, for it was found that nearly all carnation plants, whether grown in the greenhouse or out of doors, in pots or in beds, showed more or less evident traces of the disease when examined critically.

It was thought by Arthur and Bolley that the germs gained entrance to the tissues through the stomata, and sometimes through insect punctures, especially those made by aphides. They say that "the common green fly, or aphis, of the greenhouse may in some instances prove such an efficient bearer of the contagion that every leaf on a plant may be inoculated at hundreds of points, and the whole plant be turned a sickly yellow by the growth of the bacteria in the tissues."

The evidence of these infection experiments, so far as reported by Arthur and Bolley, is rather in favor of the bacterial nature of the disease, but the experiments they instituted were too few and there is no report of the isolation of the germ from spots produced artificially.

Besides the supposed production of the disease in the carnation, these investigators state that it can be transferred also to "*Dianthus caryophyllus*, *D. plumarius*, *D. japonicus*, *D. chinensis*, and *D. barbatus*, but not to the shoots, leaves, or tubers of potato or to other non-caryophyllaceous plants." These apparently successful infection experiments can be readily explained by assuming that the leaves of the inoculated plants had been previously punctured by aphides. If the leaves were not making a rapid growth they would appear to be perfectly healthy, showing no spots for some days after being punctured, as will be explained farther on. This fact not being known at the time it was almost impossible to avoid mistakes.

INVESTIGATIONS OF THE DISEASE IN WASHINGTON.

PRELIMINARY STUDIES.

In 1897 the plants in one of the large carnation houses belonging to the Government Propagating Gardens were badly affected with the disease. They were making poor growth, and the leaves were filled with innumerable translucent spots and blotches, and in many cases were deformed and twisted. The house had been kept rather dry, and in watering the plants the foliage had been wet as little as possible. It was not clear, therefore, how bacteria could have developed in sufficient numbers on the surface of the dry leaves to gain entrance to the tissues.

An extended experiment was carried on with different germicidal substances. One block of the plants was thoroughly sprayed with corrosive sublimate solution (1 to 1,000). It was very difficult to wet the foliage, but by using a fine spray much of the fluid remained attached to the surface of the leaves. In order to make the test more thorough, the waxy bloom on the younger leaves of some of the plants was removed with cotton moistened in the corrosive sublimate solution, special care being taken to disinfect the young growing point as

thoroughly as possible in this way. A similar experiment was made with formalin 1 to 1,000, and a third one with formalin 1 to 500, it being thought that the formalin would be effective in penetrating between the young leaves and thus destroying the germs. Although these applications were continued twice a week for a month, the young growth continued to be as badly diseased as ever, there being no apparent difference between the treated and the untreated plants. A few thrips were observed working on the plants and also a few aphides, but it was not believed at the time that these could possibly account for the trouble, and they were looked upon simply as distributers of bacterial infection.

Finally it was decided to make no further attempt to control this disease, but to keep the houses a little warmer and moister, force the flowers into bloom, and get as much out of them as possible. The plants were therefore syringed on bright days and the air of the house kept quite moist. Much to the writer's surprise, they began to grow out of the trouble and produced some fine flowers. These peculiar results, together with the similarity of the disease affecting the lily, as before stated, led to a careful study of the trouble.

MICROSCOPICAL INVESTIGATIONS.

Besides the affected plants in the carnation houses at Washington, other plants showing the trouble were obtained from many of the large carnation-growing sections, including some plants that Dr. Arthur kindly sent in from Mr. Fred Dorner's place at Lafayette, Ind. Leaves showing various stages of the disease were killed by both the chromic acid and the absolute alcohol method, and then were dehydrated with alcohol and infiltrated with paraffin in the usual way. Microtome sections were then cut and mounted in series, and these were stained with Ziehl's carbol fuchsin, and also, according to Gram's method, with aniline water gentian violet. The ordinary aqueous solutions of fuchsin and gentian violet were also tried along with other stains. About two thousand sections, representing all phases of the disease from different localities, were prepared and stained.

The cells of the diseased spots were found to be much larger than normal, and thin-walled and œdematous (figs. 1, 2, and 3, and Pl. III, figs. 1, 2, 3). In the early stages of the disease the chloroplasts were undeveloped or smaller than in the healthy cells and were colorless or yellow. Even after the most thorough and careful staining no parasitic or saprophytic organisms could be detected in the tissues of these spots until after the epidermal cells collapsed, when, in some cases, fungi and bacteria were readily distinguished, although usually only in small numbers. A very curious structure was always present in the earlier stages of the common form of the disease. It stained slightly with carbol

fuchsin and gentian violet, and reminded one of a very thick, resistant, slightly branched mycelium (figs. 2 and 5, and Pl. II, fig. 4). In the later stages it was found to break up, the pieces remaining for some time between the cells (fig. 2). This was later shown to be the substance left by aphides in puncturing the tissues, and will be described more in detail farther on. Nothing of this kind was found in the form of the disease characterized by the minute spots, which do not increase much in size, or in the elongated spots with irregular outlines.

The only bodies in the diseased cells that might be mistaken for

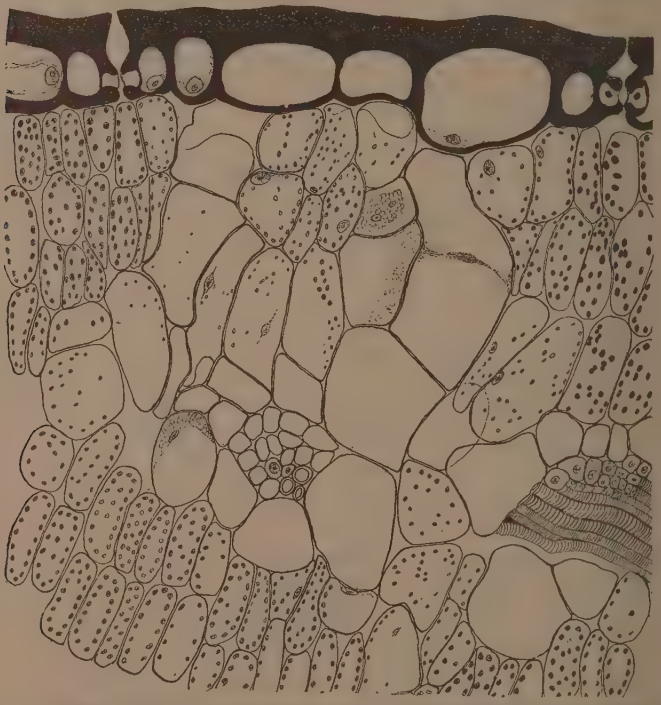

FIG. 1.—Cross section through a fully developed spot in a mature carnation leaf, the section being cut a little to one side of the lines of puncture. The diseased cells are greatly enlarged and have lost most of their chloroplasts. (Drawn with Zeiss camera lucida, × 264 diameters.)

bacteria were small proteid granules, resembling proteosomes, which stained with difficulty, swelled up, and lost their shape in 5 per cent potash. The chloroplasts of the diseased cells either do not develop at all or after having lost their color shrink to about half their normal size, and, like the proteosomes, stain with difficulty.

The microscopical examination therefore indicated that the disease could not be attributed to bacteria, for had these been present in the cells in considerable numbers they would have been readily detected, especially in the stained material.

Microscopical examination alone is of course not sufficient to absolutely settle a point of the kind in question. The tissues must be carefully examined according to the best culture methods. After many trials in washing and flaming the leaf it was found that it was impossible, as before stated, to free the cuticular portion from saprophytic organisms without heating the leaf to such an extent that

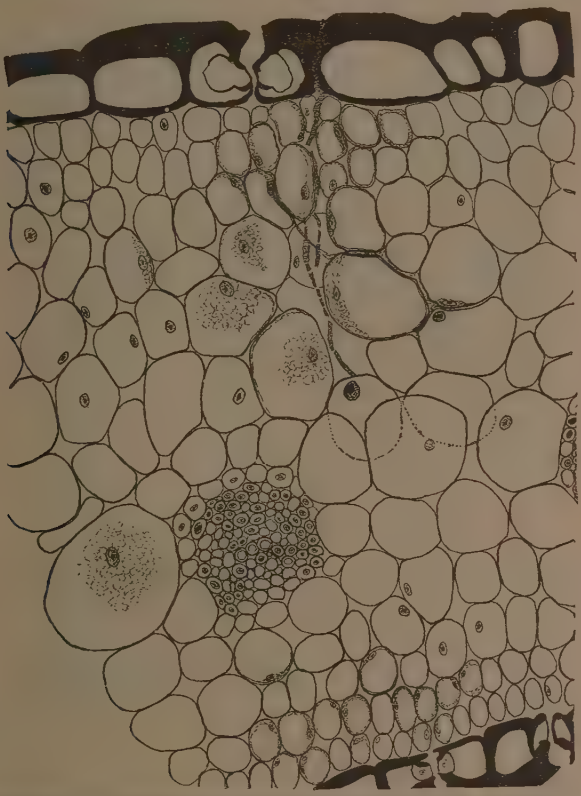

FIG. 2.—Cross section of a carnation leaf several days after it had been punctured by an aphid. The proteid sheaths between the cells mark the line of puncture. The cells on both sides have become abnormally large. (Drawn with Zeiss camera lucida, × 248 diameters.)

internal forms would also be destroyed. In cases where heating had not been continued long enough to kill the epidermal cells, surface cultures of the cuticle of flamed leaves often developed both molds and bacteria. Cultures were therefore made from the diseased mesophyll direct by carefully peeling off the epidermis of the leaf and scraping out the inner tissues with a flamed scraper which had been allowed to become perfectly cool. After the removal of the epidermis

in this way—a very easy process, requiring no cutting, except enough to start the peeling—the diseased spots could be as readily detected as before. From one to twenty spots, varying in size from 0.5 to 2 mm. in diameter, were included in each culture. The cells were broken up as much as possible in scraping them out of the tissues, but great care was taken not to allow the internal tissues to become contaminated in any way.

During the first season's work about five hundred cultures were

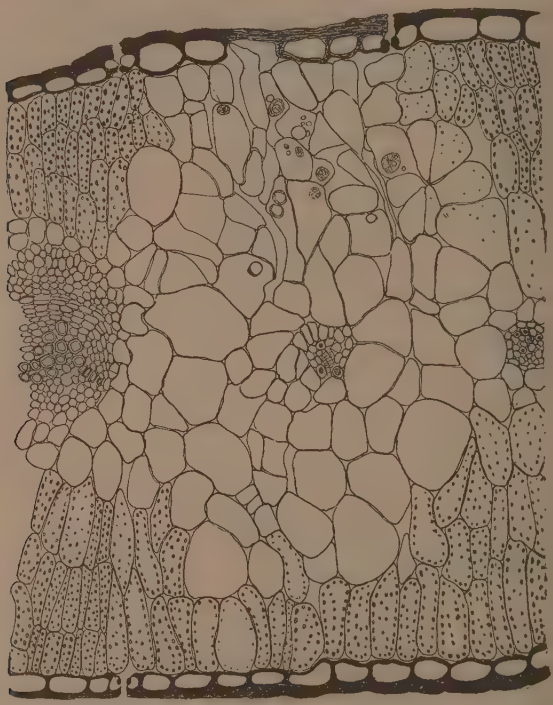

FIG. 3.—Cross section through the middle of a fully developed spot on a mature carnation leaf. All the epidermal cells in the spot have collapsed. Most of the enlarged diseased cells have lost their chloroplasts, and many of them contain globular vacuolate masses, which are portions of the disorganizing cell structure. (Drawn with Zeiss camera lucida, × 135 diameters.)

made, various media being used—for instance, slightly acid, neutral, and slightly alkaline beef broth, with and without peptone; potato broth of various strengths; cauliflower broth; potato cylinders; agars of various composition; gelatin (acid, neutral, and alkaline to litmus and to phenolphthalein), etc. These cultures included many poured plates made direct from the crushed tissues. In no case, however, were organisms found in any cultures made from a spot before the epidermal tissues had collapsed.

In preparing the material for the cultures, only that known to

be free from outside contamination was used. Whenever there was the slightest doubt as to its purity it was either discarded or the tubes were marked "contaminated." Frequently tubes marked in this way, however, remained free from any organism. In cultures made from spots which had collapsed various fungi and bacteria were occasionally obtained, but this was not constant nor were the organisms always of the same sort. Cultures including the epidermis frequently contained various organisms, among these being a yellow bacterium, which occurred frequently on the surface of both diseased and healthy leaves. The morphological characters of this bacterium and its growth in the various media, with the exception of acid gelatin and agar, agreed well with *Bacterium dianthi* as described by Arthur and Bolley. On the two media mentioned the colonies were surrounded by a peculiar light band, which was at first thought to be some contaminating organism, its structure being like exceedingly minute cocci, the largest less than five-tenths of a micron in diameter. Attempts were made to cultivate this supposed organism on various media, but all failed, and even with the two media mentioned it was not possible to obtain a colony of the yellow germ which was not accompanied by this peculiar external formation. On neutral or alkaline media, however, no such change took place. Further investigation showed that these coccus-like bodies were made up of something precipitated from the culture medium by the alkali which the germ produced. Nothing of this kind was described for *Bacterium dianthi* by Arthur and Bolley.

INFECTION EXPERIMENTS IN 1897.

As the orange yellow germ agreed in most respects so closely with *Bacterium dianthi* and as no other yellow organism that could possibly be confused with *B. dianthi* was found in the hundreds of leaves studied, it was determined to make special use of this germ for infection experiments, but several other common forms were also used, the principal one among these being a white organism which liquefied gelatin readily.

Great difficulty was experienced in finding healthy plants with which to work. Those finally selected were taken into the laboratory, thoroughly freed from aphides and thrips, and allowed to grow under moist bell jars for three weeks. During this time the disease developed in some of the leaves which were apparently healthy when brought into the laboratory. The spots in such case were marked with india ink so that they would not be confused with spots that might result from infections. After the period of incubation had passed and a series of plants had been obtained which were reasonably free from spots, and also from aphides, thrips, and red spiders, rapidly developing fluid cultures of the germ were thoroughly brushed into the surface of the

youngest unfolded leaves and also into the healthy portions of the older ones. Besides brushing in the cultures, abrasion inoculations and also hypodermic injections with an ordinary hypodermic syringe were made.

Ten plants were thus treated with *B. dianthi* during the season of 1897, the three methods of infection being followed in the case of different branches of each plant, but the disease was not produced in any case. An occasional spot developed on some of the plants after treatment, but such spots were always close to a spot which had been previously marked with India ink, and so could not be attributed to infection by the germs applied. Negative results also were obtained with the white germ mentioned.

Further infection experiments with *B. dianthi* were carried out in 1898, and these will be described later on in this bulletin. The results of the writer's work so far showed no evidence that the disease was caused by bacteria, and a search was therefore instituted for other possible external causes.

FIRST COLONIZATION EXPERIMENTS WITH APHIDES.

Arthur and Bolley regarded aphides as "such efficient bearers of the contagion that every leaf on a plant may be inoculated at hundreds of points," and indeed the writer observed that the spots were extremely common on many plants which had been attacked by the aphides. A careful study revealed the fact that carnations are seldom, if ever, entirely free from aphides—they may often be found between the young appressed leaves of plants apparently free from them. Ten young plants were taken into the laboratory and entirely freed from the insects by first picking off all that could be found, and then fumigating the plants lightly with hydrocyanic acid gas. The plants were kept under moist bell jars, and as soon as growth free from spot had developed aphides were colonized on various leaves. In the case of the youngest, most rapidly growing leaves spots could be detected with a hand lens three days after the tissues had been punctured by the aphides; in leaves somewhat older a longer time elapsed before the spots became visible; while in leaves half size, especially if the plant was making slow growth, the spots could not be seen for about two weeks after the puncture was made.

The spot appeared first as a minute translucent dot, accompanied by a slight swelling of the tissues. On some of the very young leaves the spots were unusually large, being from 2 to 3 mm. in diameter by the time the leaf had reached full size. Sections were cut from the spots so produced and were stained as before described, but no parasitic organisms could be found in the tissues. The cells had enlarged and the chloroplasts had lost their color and had failed to develop or had shrunk in the manner characteristic of the disease. The peculiar

mycelial-like structure believed to be left by the aphis was always present, extending from the surface of the leaf down to the soft bast of the vascular bundle, and often beyond into the central mesophyll portions of the leaf (figs. 2 and 5, and Pl. II, fig. 4). Large numbers of cultures were also made from spots known to be produced by aphides, but with the same negative results as before described.

Similar experiments with thrips showed conclusively that they are the cause of the elongated spots (fig. 4, and Pls. I and III) and the distortion of the foliage, and it was also found that the red spiders working on the immature leaves produced the very minute spots by sucking the nourishment from the epidermal cells and those immediately underlying.

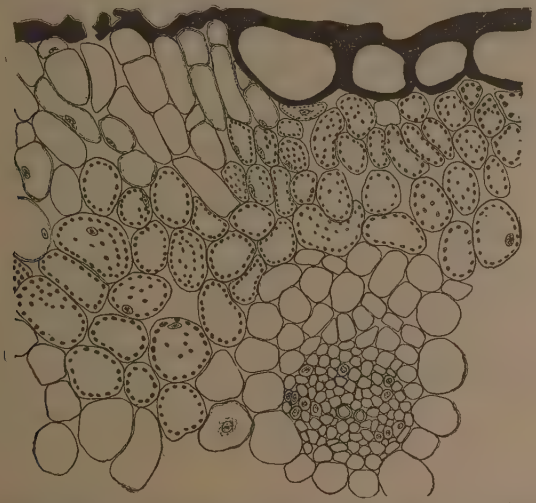

FIG. 4.—Cross section through a fully developed spot produced by thrips. The epidermal cells have collapsed and the underlying palisade parenchymal cells are somewhat elongated and have lost most of their chloroplasts. (Drawn with Zeiss camera lucida, × 282 diameters.)

It was thought desirable to get more accurate information in regard to the manner in which aphides obtain their nourishment from the tissues, and for this purpose leaves upon which they had been colonized were carefully cut from the plant while their sucking apparatus was still inserted in the tissues. These leaves were dropped into 75 per cent alcohol saturated with corrosive sublimate, and many of the aphides were thus killed before they had time to withdraw their beaks or puncturing bristles. Portions of the leaf with the beaks attached were then cut out, carefully dehydrated, and infiltrated with paraffin, the sections being then cut and mounted in series, and thus preparations showing the puncturing apparatus in place in the tissues obtained (fig. 5, and Pl. II, figs. 2 and 3). The puncturing bristles always

extended at least to the soft bast, that is, nearly halfway through the leaf. The proteid sheath secreted by the insect while inserting its puncturing bristles was well stained with carbol fuchsin and gentian violet by allowing the sections attached to the slide to stand in the stain at a temperature of 55° C. for two hours. A slight and transient staining is thus obtained in fifteen minutes.

These sections showed, as Büsgen[1] pointed out in the case of many other plants, that the puncturing apparatus does not lacerate the cells, but passes between them, curving around obstructions and following the middle lamella as shown in the figures cited, the gelatinous sheath being secreted from the mouth parts of the insect as a support for the fine bristles as they wedge their way between the cells. The tip of the puncturing apparatus stops in the region of the elongated soft bast cells, which it punctures and from which the proteid contents are removed. Sugar is also sucked out of the cells in this region, passing through the alimentary canal and appearing on the plant as honeydew. It is probable that part of the sugar is used as food by the insect, but the larger part is excreted, while most of the proteid substances are retained and evidently constitute for the insect the principal food element extracted from the tissues.

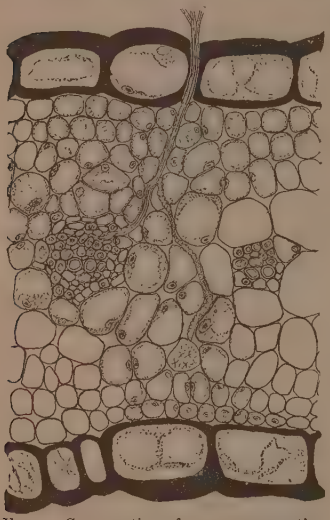

FIG. b.—Cross section of a young carnation leaf showing an aphid's sucking apparatus in the tissues. The tip of the beak is in the soft bast of the vascular bundle. The first puncture passed to the right between the two bundles, and after the withdrawal of the beak from this region the proteid supporting sheath secreted by the insect remained between the cells. No apparent change has yet taken place in the cells. (Drawn with Zeiss camera lucida, × 225 diameters.)

INFECTION AND COLONIZATION EXPERIMENTS IN 1898.

The experiments described proved pretty conclusively that the disease in question must be attributed to the punctures of insects and mites, it having been shown definitely that plants kept free from these animal organisms remained perfectly free from the disease, and that when they were present it invariably developed. In order to settle the matter beyond any possible doubt, colonization experiments with insects were carried on under perfectly antiseptic conditions and inoculation experiments made with bacteria upon plants which had been kept absolutely free from puncturing insects and mites.

[1] Büsgen, M., Der Honigtau. Biologische Studien an Pflanzen und Pflanzenläusen, Bes. Abdr. a. d. Jen. Zeitsch. für Naturwis., Bd. XXV, N. F. Bd. XVIII.

For these experiments young rooted cuttings of the Scott, McGowan, and Garfield carnations, as free as possible from the disease, were obtained, 50 of each variety being set out in a small house, in which the conditions could be easily controlled, and 50 each in a larger house. In addition to these, seedlings of as many of the species and varieties of Dianthus as could be obtained were also grown, one lot in the small and the other lot in the larger house. The plants in the small house were fumigated with hydrocyanic acid gas and tobacco, and were carefully examined once a week to see that they were perfectly free from aphides, thrips, and red spiders, while on the other hand the corresponding lots of plants in the larger house were colonized with aphides and thrips.

All the young growth that developed in the small house during two months remained free from the disease, although the house was constantly shaded and half of each lot of the plants were syringed each day overhead—a treatment supposed to favor the development of the disease. It was found that those which were syringed were, on the whole, a little better developed than those left dry, but otherwise there was no difference. In the larger house, however, a marked difference was apparent at the end of this period between the two sets of plants, those which had been syringed overhead showing 50 per cent less spot than those kept dry. This difference is readily explained by the fact that the syringing prevented the excessive multiplication of aphides and the increase of red spiders, and also drove off many of the thrips. Had bacteria had anything to do with the disease the plants syringed overhead should have suffered most. Not only did the carnations become spotted from the punctures of aphides, thrips, and spiders, but all the varieties of Dianthus reacted in a similar manner. The thick-leaved forms developed spots much like those on the carnation, while on the thin-leaved forms the spots dried out more quickly, and the leaves showed greater distortion when attacked by thrips.

BACTERIAL INOCULATIONS.

At the close of the experiments above described the plants in the small house, having an abundant new growth and being absolutely free from insects and mites, were in prime condition for bacterial inoculation experiments. *Bacterium dianthi* from a fresh, rapidly growing liquid culture was therefore inoculated into ten plants of each of the three varieties of carnations mentioned; three plants each of *Dianthus arenarius*, *D. chinensis* (two varieties), *D. caesius*, *D. barbatus*, and *D. plumarius*, and sixteen horticultural varieties of these species; and five varieties of seedling carnations—a total of 6 species and 26 varieties, including the three varieties first named (111 plants in all). All the young growth, including many of the mature leaves, was thoroughly washed with water and beef-broth solutions containing the germs.

Besides the surface application of the germ, many slight abrasions with a fine needle were made on the surfaces of the inoculated leaves of three plants of each of the first three varieties of carnation mentioned. The house was kept shaded and the surface of the leaves was moistened each day by a light sprinkling in order to give the germs a good opportunity to develop, but not a single spot that could be attributed to the bacterial inoculation developed on any of the plants during a period of six weeks. The abrasions in many instances showed swollen edges and became slightly yellow, as in the case of the abrasions described by Arthur and Bolley. They did not increase in size and could not be attributed to the action of the germ, especially as the abrasions made on the control plants behaved in exactly the same manner.

ANTISEPTIC COLONIZATION.

Four plants of each of the three varieties of carnations, or twelve plants in all, were taken into the laboratory at the beginning of the inoculation experiments and were placed under large bell jars. These plants had a new growth which had never been injured by insects or spiders and were perfectly free from spots. One plant of each variety was used for a control experiment, no aphides being permitted on them; aphides were colonized on another lot, also consisting of one plant of each variety; and every leaf of another lot, consisting of two plants of each variety, was carefully washed with corrosive sublimate solution, 1 to 1,000 parts of water, all the glaucescence being carefully removed from the leaves and stems, it being easy in this way to determine when the plants were thoroughly wet and consequently disinfected. Previous to this a colony of aphides (*Rhopalosiphum dianthi* Schrank) had been grown upon a similarly sterilized plant until there were about 300. Out of this number 25 were selected at random, mashed up, and cultures made from the mass, but these cultures were perfectly free from bacteria or fungi. Before colonizing the other aphides they were allowed to crawl over the surface of moist agar in test tubes to determine whether they were free from bacteria, and as none of the latter developed, it was known that each individual used in the colonization experiment was free from germs.

The experiment was begun May 17, 5 aphides being put on each plant except the controls. On May 20 several spots could be plainly seen in the region where the aphides had been working on the most rapidly growing young leaves, and from this time on numerous spots appeared. On May 24, just one week after the aphides had been colonized, there were on one of the sterilized plants 30 aphides and 250 spots could be seen with the aid of a hand lens, on another there were 20 aphides and 170 spots, on a third 20 aphides and about 170 spots, and on a fourth 40 aphides and about 250 spots. The other two plants

of the sterilized series and all the unsterilized plants except the controls kept free from insects, showed an equally rapid development of aphides and spots. After this no exact account was kept of the rapidity of increase of aphides or spots in any case, but the young leaves, as well as those which had matured, and also the stems, were completely peppered with spots, and the plants assumed a sickly yellowish appearance.

It was not thought necessary to make cultures from the spots on the sterilized plants, owing to the facts that the work had been done under thoroughly antiseptic conditions; that the entirely negative character of the previous culture work was deemed sufficient; and that the three control plants, upon which no aphides were allowed to develop, remained absolutely free from spot. The evidence of these experiments, it is believed, settles beyond any doubt the fact that the disease in question is not due to bacteria, but is the work of aphides, thrips, and red spiders.

CAUSE OF THE INCREASE IN THE SIZE OF THE SPOTS.

It may be asked why in the absence of parasitic and saprophytic plant organisms the work of aphides, thrips, and red spiders is followed by such marked pathological changes in the tissues, and why do the spots increase in size after the insect or mite has done its work. This question was investigated to some extent, and it is believed that an explanation is at hand. As previously pointed out, the aphides do not lacerate the mesophyll cells between which they force their sucking apparatus and excrete the supporting tube, but still the cells along the line of insertion show the change first, the chloroplasts not developing and becoming yellower and the cells becoming somewhat enlarged and œdematous.

It is difficult to determine the relative acidity of the tissues involved, the spots being small, but as nearly as could be determined, the diseased cells were apparently less acid than the healthy ones. Another difference between the diseased and the healthy cells was more marked and could be readily determined. After the epidermis of the leaf had been carefully peeled off, the surface of the exposed mesophyll cells was moistened with a 2 per cent solution of gum guaiac in absolute alcohol, and in a moment or two the diseased spots became a deep blue, while the surrounding healthy cells showed but a slight reaction, which could be detected only by the apparent deepening of the green. This test was applied in still another manner. Diseased tissues removed from the leaf were first treated with 95 per cent alcohol and then transferred to a small amount of distilled water, in which they were crushed and allowed to stand for half an hour. The solution was then tested with guaiac tincture, and for comparison an equal amount

of a solution made similarly from healthy tissue of the same leaf was also tested in like manner. The action of the diseased extract on gum guaiac was about twice as strong as that of the healthy extract. It seems very probable that this remarkable increase in the oxidizing power of these cells must be the result of some irritating substance, perhaps either an organic acid or some other substance from the mouth parts of the insect which it injects into the tissues at the time it punctures them. It may be that the increase of the oxidizing enzym in the cells is an attempt on the part of the latter to destroy the injected irritant.

As the writer has shown in his paper on the relation of oxidizing enzyms to the destruction of chlorophyll,[1] the destruction of the chloroplasts can probably be explained by the increased oxidation taking place in the cells. The nucleus of the cells involved is not destroyed in the earlier stages of the trouble. The nutrition of the cell, however, is interfered with and the chloroplasts do not develop normally. As before pointed out, the rapidity of the increase in size of the spots on the young leaves is coordinate with that of the growth of the leaf. In a mature leaf, however, the size of the spots may increase very slowly, and this must be due to either a gradual diffusion of the irritant injected by the insect or to the direct effect of diseased neighboring cells.

The former supposition is more likely to be correct, as the same kind of a mechanical wound is followed in the case of different insects by markedly different results. For example, thrips, aphides, and the ordinary little green leaf hoppers found on *Chenopodium album* produce only a slight translucent spot on the leaves, this developing only after a week or ten days from the time the puncture is made. On the other hand, a little reddish leaf hopper, which is usually found working on the lower surface of these plants, produces in eighteen hours from the time a puncture is made a purple spot 1 mm. in diameter which rapidly increases until it is 4 to 5 mm. in diameter. Evidently this insect injects some quite rapidly diffusing substance into the tissues.

The writer has carried out careful colonization experiments with this red leaf hopper and has endeavored to determine what the injected substance is, but so far has not settled this point. In the case of the Bermuda lily, a great difference has been noted in the relatively slight injury produced by the common green aphis, *A. maidalb*, and the greater injury produced by the large green aphis belonging to the genus Siphonophora. The same is true of the brown or black aphides and the green aphides which attack the violet, the former producing a marked stunting of the plants and the latter doing but little injury outside of distorting the flowers. The work of the San José scale on

[1] Centralbl. f. Bakt., 1899, Abt. 2, Bd. V.

the apple and pear as compared with the work of other scale insects is also a case in point, the increase in the oxidizing enzym which follows its attack being enormous and the evident injury spreading frequently 6 to 8 mm. from the point of puncture.

Besides the irritants injected and the consequent changes which take place in the neighboring cells, account must be taken of the elaborated plant food extracted by the insects in question, as the waste of saccharin materials in the form of honeydew is often enormous, and the proteid material used by the insects themselves must be quite considerable, especially when they are numerous. The stunting of the plant is probably in part due to the loss of food material in this way.

The work of aphides, thrips, and red spiders often produces in other plants changes similar to those following their attacks on carnations. This is especially true of rather thick-leaved plants, such as Bryophyllum, tulip, etc. It was found that as a rule plants rich in oxidizing enzyms react more strongly to punctures of this kind, and that plants which have been weakened by long-continued forcing, and consequently have made a poor, starved growth, are much richer in these oxidizing enzyms than are the stronger-growing, more vigorous individuals of the same variety. It is also an interesting fact that the aphides are especially fond of these weak plants and increase rapidly on them.

EFFECT OF THE DISEASE ON THE PLANT AS A WHOLE.

The effect of the disease on the plant as a whole depends upon the individual vigor of the plant and upon the number of punctures. A few punctures made by aphides, thrips, or red spiders will not seriously injure a plant, but a large number will cause it to become prematurely stunted and yellowish and also more susceptible to injury from various causes, especially parasitic fungi, which attack the weak stems and roots. Thrips, even though not numerous, may often cause the leaves of healthy, vigorous plants to become much distorted and show light blotches where they have been punctured. The value of flowers punctured in this way is of course materially reduced.

Red spiders seldom do much injury unless the foliage of the plants has been kept very dry. The stunting of the plants which follows a severe attack of any of these animal organisms brings about a premature ripening of the lower leaves, especially if the plants are rather crowded, and such leaves gradually turn yellow and die. Any other cause which stunts the plants growing under the conditions mentioned, however, will bring about a similar maturing and death of the lower leaves.

The susceptibility of different varieties of carnations to the injury seems to be as a rule proportionate to the normal vigor of the variety under the conditions in which it is growing when attacked. The

constitutions of certain varieties seem to be very sensitive to injuries of this kind, while others are not, and while the sensitiveness, or resistance, as the case may be, is influenced to a large extent by the conditions of growth and the manner of propagation and selection of cuttings, yet this natural inherent constitution of each variety is the only explanation for the different degrees of susceptibility of different varieties.

Among the varieties which the writer has had under observation, Uncle John, Alaska, Della Fox, and McGowan as a rule proved to be very sensitive to the punctures of not only aphides, but also to those of thrips and red spiders. Vigorous plants of the variety Storm King spot readily as the result of the punctures of aphides, but do not appear to be otherwise injured much, while weak plants of this variety become badly stunted and lose many of their leaves. The same may be said of Bridesmaid, Sweet Brier, Scott, Garfield, Daybreak, Meteor, and Jacqueminot. Garfield and Daybreak are much more injured by thrips than by aphides. Among the other species of Dianthus, *D. arenarius* is particularly sensitive to aphis punctures, especially if the stem is much injured, in which case the plants often die. *D. fanatica*, *D. caesius*, *D. chinensis*, *D. atrobens*, and *D. plumarius* are less sensitive, but spot readily from punctures of aphides, thrips, and red spiders. *D. chinensis heddewigii* and its varieties, especially the thin-leaved sorts, are particularly sensitive to the punctures of aphides and thrips, and are quite sensitive also to the punctures of red spiders.

METHODS OF CONTROLLING THE DISEASE.

As the result of their investigations, Arthur and Bolley recommended that aphides be kept down as thoroughly as possible and that particular care be taken not to wet the foliage in watering. These recommendations were based on their theory that the aphides serve in a measure as carriers of the disease and that water facilitates the development of the bacteria that were believed to be the cause of the malady.

There is much to commend in the methods of watering suggested by Arthur and Bolley, and their efforts in stimulating search for better methods of staking the plants so as to keep the foliage more exposed to light and air have also resulted in much benefit. The increased vigor resulting from the use of these improved methods, however, was not due to a decrease in the number of spots, as was first believed, but to the fact that the total injury resulting from punctures was reduced.

The genus Dianthus is adapted to dry situations,[1] but all the species

[1]Williams, F. N., Jour. Roy. Hort. Soc., 1891, Vol. XII, p. 464; Arthur and Bolley, l. c.

are nevertheless often exposed to drenching rains when growing under natural conditions, and the thick-leaved varieties are well adapted to shed water. Growers generally believe that the proper syringing of plants with water under a pressure of 20 to 25 pounds is valuable, and the writer's observations lead him to the same conclusion. It must be remembered, however, that this work should be done in bright, clear weather, so that the foliage will dry thoroughly before night. Proper syringing of plants instead of favoring stigmonose is an important factor in holding it in check, as it keeps down red spiders, and in a measure helps to reduce the number of aphides, thrips, and similar insects. To keep down aphides, however, tobacco in its various forms must be mainly relied on. Carnation growers are so familiar with the methods of using tobacco that nothing need be said upon this subject here. It must be borne in mind, however, that healthy plants can not be obtained unless the insects and spiders are prevented from getting too much of a start, hence fumigation and syringing should be so timed and so carried on that these pests will be held in check.

The various forms of tobacco extract used in liquid form, and also when evaporated by means of hot irons, bricks, etc., have certain advantages, chief of which is that the flowers are less injured by the disagreeable odors. Hydrocyanic acid gas has been found effective, but it can not be recommended unqualifiedly, owing to the fact that it is likely to injure certain varieties. Scott, Garfield, Meteor, and McGowan can stand one-tenth gram of 98 per cent potassium cyanide per cubic foot of space for fifteen minutes without material injury. This will kill about 90 per cent of the aphides, but will not kill thrips or spiders. A stronger dose of gas or a longer exposure can not safely be recommended.

It has been found that different plants of the same variety react very differently to the punctures of both insects and spiders, and this shows the importance of a rigid method of selection for the purpose of building up a vigorous strain of plants. In propagating plants preference should always be given, other things being equal, to those which show the least evidence of stigmonose. Some plants will be found almost entirely free from it, and it would probably be well to start selection work on a small scale from such stock and continue it from year to year until a more resistant strain is obtained. The writer is satisfied that such a practice would result in much benefit at once, and that within three to five years the vigor of the stock might be materially increased.

CONCLUSIONS.

(1) The disease of carnations characterized by the symptoms described in this bulletin is widespread, and under certain conditions unfavorable to the plant it is quite injurious.

(2) So far as can be determined by the most careful microscopical study and bacteriological tests, neither fungi nor bacteria are present in the earlier stages of the disease.

(3) As the disease progresses various fungi and bacteria may appear, but their presence is not constant.

(4) Infection experiments with bacteria and fungi, especially with the germ described as *Bacterium dianthi*, carried out under the most rigid bacteriological conditions, resulted negatively in every case.

(5) A disease having all the characteristic symptoms of the so-called "bacteriosis" except the presence of bacteria, is produced by the punctures of aphides, as was repeatedly demonstrated by colonizing these insects on carnations.

(6) That aphides and not bacteria are responsible for the trouble is shown by the fact that the injuries produced are not accompanied in the earlier stages by fungi or bacteria. The aphides therefore can not be looked upon as simply carriers of some fungus or bacterium, as they produced the disease on plants growing under perfectly antiseptic conditions as quickly as upon those not protected by antiseptics.

(7) Injuries similar in many respects to those produced by aphides also result from the attacks of thrips—insects which are often present on carnations growing under glass, although sometimes overlooked by growers. Another form of the disease is produced by red spiders.

(8) No matter how badly diseased plants may be, if otherwise vigorous they will grow out of the disease entirely and the young leaves and shoots will remain free from spots if kept completely free from aphides, thrips, and red spiders.

(9) As the disease is not due to bacteria the name "bacteriosis" is inappropriate and therefore stigmonose is suggested for the trouble.

(10) The carnation is readily influenced by the conditions under which it is grown, and as a result its reaction to the injuries of the aphides, thrips, and spiders, and its susceptibility to their attacks, not only varies in different varieties, but also in individuals of the same variety. Plants grown under improper conditions, therefore, show more of the characteristic injuries from a given number of punctures than do plants growing where all the conditions are favorable. Certain plants rich in oxidizing enzyms have been shown to react more quickly to the work of puncturing insects and mites than plants poor in these enzyms.

(11) The size of the spots made by the punctures of aphides increases in proportion to the rapidity of growth of the leaf and the susceptibility of the plant, and also depends to some extent on the genus and the species of insect which makes the puncture. It is believed that the insect injects some irritating substance of an acid or enzymic nature into the wound, that this substance causes the increase of oxidizing enzyms in the cells which it reaches, and that these enzyms

interfere with the nutrition of the cell by destroying the chlorophyll and setting up other changes which finally result in death.

(12) Besides the carnation and different species of Dianthus, many other plants react similarly to the puncture of aphides and other sucking insects, and also to mites.

(13) The grower can successfully combat this disease by the proper selection of cuttings; careful propagation of stock; good soil; the proper amount of moisture, light, and air; and by the reduction of aphides, thrips, and red spiders to a minimum.

EXPLANATION OF PLATES.

PLATE I.—Spots produced by aphides and thrips. Figs. 1, 2, 3, and 4 show the early, intermediate, and late stages of the disease produced by aphides, as seen when examined by transmitted light. (Natural size.) Figs. 5, 6, 7, 8, and 9 show the distortion of the leaves and the spots produced by thrips. (Natural size.)

PLATE II.—Photomicrograph of sections to show early stages of stigmonose produced by aphid punctures. Fig. 1 shows a section of a healthy mature leaf. The chloroplasts show as small dots lining the palisade parenchymal cells. There are very few in the large water storage cells surrounding the vascular bundles in the middle of the leaf. (× 30 diameters.) Figs. 2 and 3 show the sucking apparatus of two aphides passing between the epidermal and mesophyll cells of a young leaf to the soft bast of the vascular bundle. The aphides were killed on the surface of the leaves and the section cut after dehydration and infiltration with paraffin. (Fig. 2 × 120 and fig. 3 × 70 diameters.) Fig. 4 shows the proteid sheath secreted by an aphis while inserting its sucking apparatus in the tissues of a young leaf. A part of another sheath is shown in the lower right-hand portion of the section. (× 200 diameters.)

PLATE III.—Photomicrograph of sections to show later stages of stigmonose produced by aphid punctures and thrips. Fig. 1 shows a cross section illustrating enlargement of cells and loss of chloroplasts on both sides of the line of puncture. (× 75 diameters.) Fig. 2 shows a more advanced stage. (× 65 diameters.) Fig. 3 shows a cross section illustrating a late stage, many of the cells being broken down and the cell contents in a number of instances forming globular plasmode structures. (× 85 diameters.) Fig. 4 shows a spot produced by thrips. The epidermal cells have collapsed completely and the cuticle has become disorganized, but still remains. Most of the immediate underlying cells have lost their chloroplasts. (× 85 diameters.)

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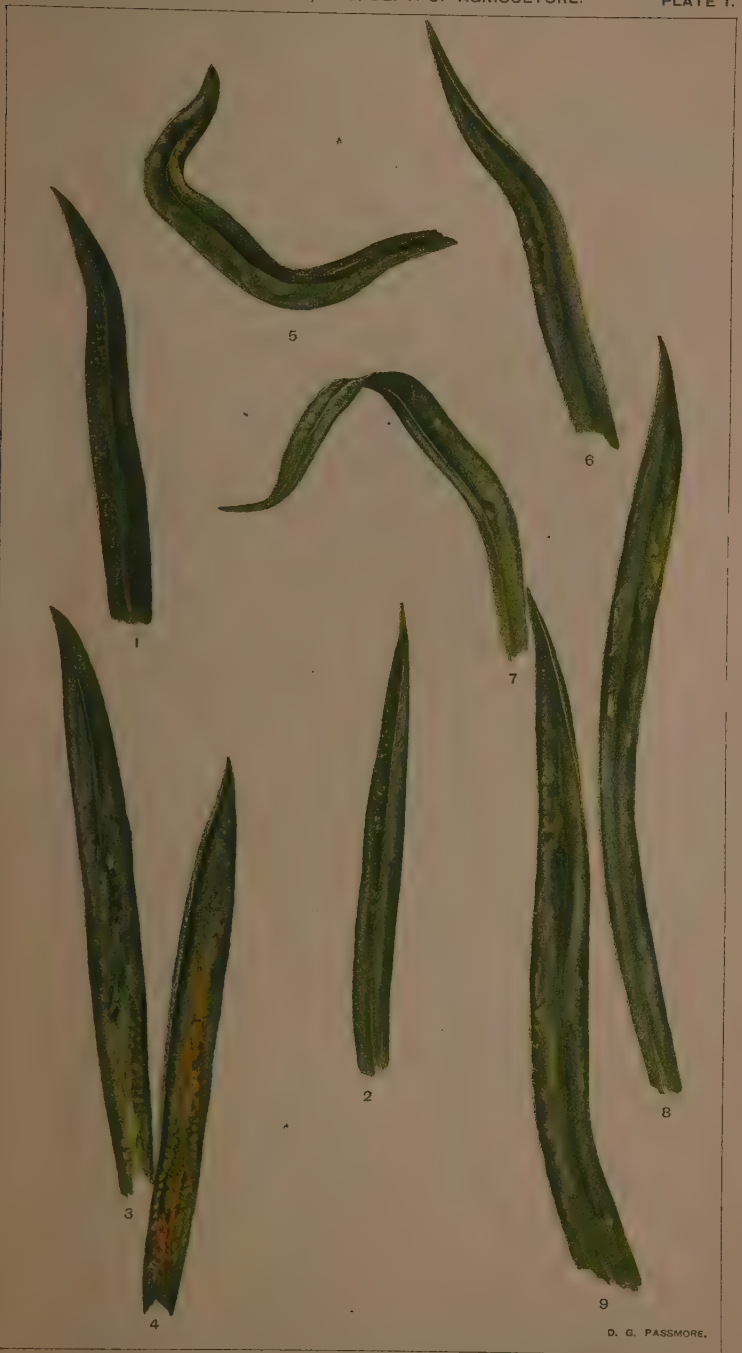

A. Hoen & Co. lith. Baltimore.

SPOTS PRODUCED BY APHIDES AND THRIPS.

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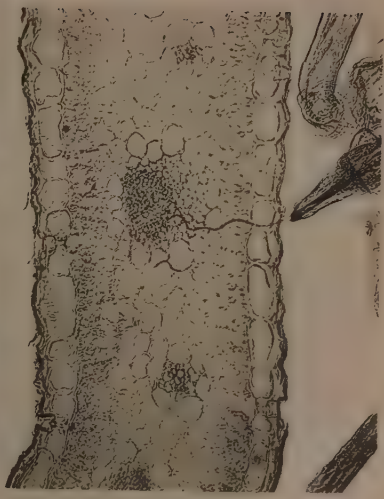

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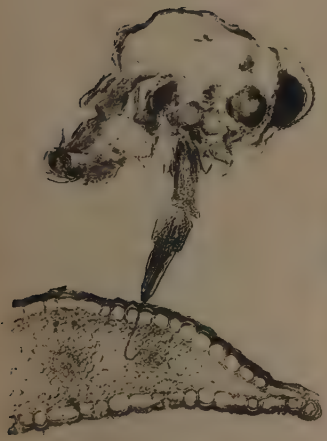

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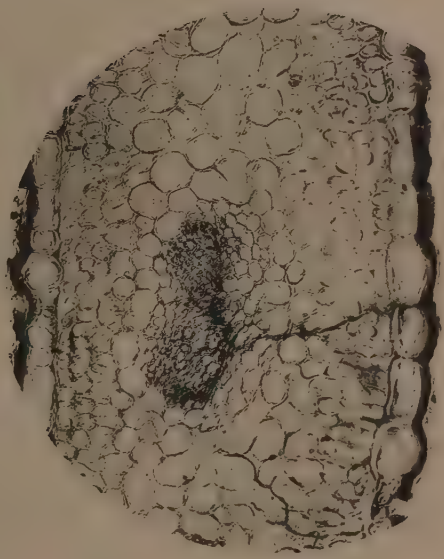

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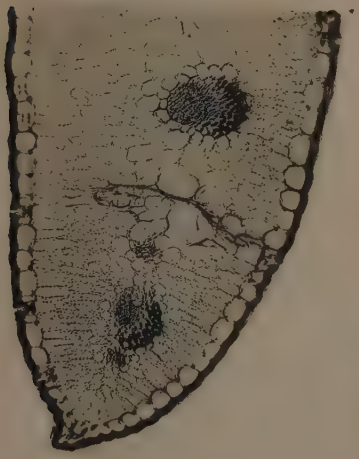
Photomicrograph of sections to show early stages of stigmonose produced by aphid punctures.



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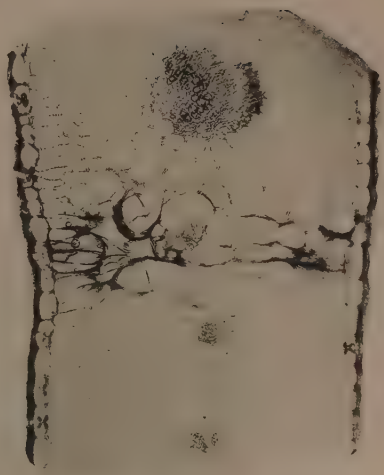

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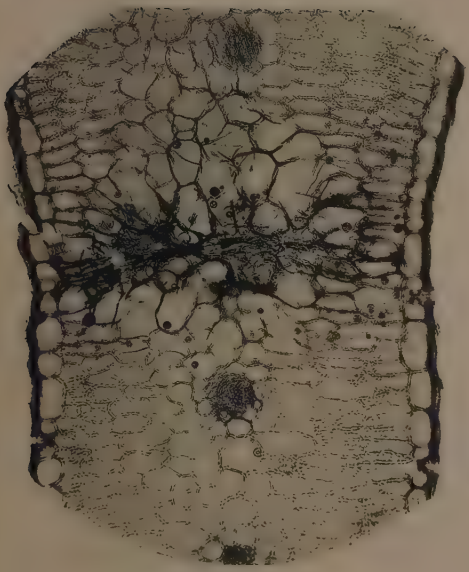

3.

4.

Photomicrograph of sections to show later stages of stigmonose produced by aphid punctures and thrips.